Community niche predicts the functioning of denitrifying bacterial assemblages

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Abstract: Predicting biodiversity effects on ecosystem functioning requires adequate evaluation of the mechanisms explaining why more diverse systems could perform better than less diverse ones. In this context, tackling functional diversity has become an important issue. Even though the aggregation of species into functional groups supposes niche differences among groups, the concept of niche has not been fully exploited in the context of the biodiversity–ecosystem functioning research. Here we report the results of microcosm experiments where we used bacteria as a model to explore whether niche differences among species provide a good estimation of community functioning. For that we used experimental communities of denitrifying bacterial species and investigated the effects of bacterial diversity on two community processes, denitrification and anaerobic CO2-production. We first measured the activities of 16 bacterial species grown individually on six different carbon sources. We then used the same set of species to assemble communities varying in both species richness and composition in microcosms containing a mixture of all six carbon sources. The performances of individual species on individual carbon sources were used to calculate, for each process measured, an a priori index called "community niche" that accounted for the performances of the species present in a given community across the entire range of the six resources. We found that species richness had a positive but small effect on both community processes whereas community niche explained a much larger proportion of the variation. According to the results of a path analysis, community niche was the main driver for the corresponding community process, but species richness affected community niche and thus had an indirect effect on denitrification and CO2 production. In addition to community niche, the presence of particular bacterial species also influenced community functioning, indicating that other effects than the capacity to use carbon sources played a, albeit minor, role in our experiment. Our study provides evidence for the importance of resource niches in shaping biodiversity–ecosystem functioning relationships of bacterial communities.

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COMMUNITY NICHE PREDICTS THE FUNCTIONING OF DENITRIFYING BACTERIAL ASSEMBLAGES

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ABSTRACT

Predicting biodiversity effects on ecosystem functioning requires to adequately evaluate the mechanisms explaining why more diverse systems could perform better than less diverse ones. In this context, tackling functional diversity has become an important issue. Even though the aggregation of species into functional groups supposes niche differences among groups, the concept of niche has not been fully exploited in the context of the biodiversity–ecosystem functioning research. Here we report the results of microcosm experiments where we used bacteria as a model to explore whether niche differences among species provide a good estimation of community functioning. For that we used experimental communities of denitrifying bacterial species and investigated the effects of bacterial diversity on two community processes, denitrification and anaerobic CO_2-production. We first measured the activities of 16 bacterial species grown individually on six different carbon sources. We then used the same set of species to assemble communities varying in both species richness and composition in microcosms containing a mixture of all six carbon sources. The performances of individual species on individual carbon sources were used to calculate, for each process measured, an a-priori index called "community niche" which accounted for the performances of the species present in a given community across the entire range of the six resources. We found that species richness had a positive but small effect on both community processes whereas community niche explained a much larger proportion of the variation. According to the results of a path analysis, community niche was the main driver for the corresponding community process but species richness affected community niche and thus had an indirect effect on denitrification and CO_2 production. In addition to community niche, the presence of particular bacterial species also influenced community functioning, indicating that other effects than the capacity to use carbon sources played a, albeit minor, role in our experiment. Our study provides evidence for the importance of

resource niches in shaping biodiversity–ecosystem functioning relationships of bacterial communities.

Key words: *biodiversity–ecosystem function relationships, community niche, carbon resource-use, bacterial diversity, denitrification, anaerobic CO_2 production*

INTRODUCTION

Identifying the mechanisms explaining why more diverse systems could perform better than less diverse ones has become an essential issue in studies focusing on biodiversity–ecosystem functioning (BEF) relationship. In this context, tackling functional diversity among species has been of great importance (Lavorel and Garnier 2002, Petchey and Gaston 2006). When aggregating similar species into functional groups, we assume that functional differences among these groups would affect the functioning of communities. Considering niche as the impact species have on resource use (according to Elton's and later MacArthur & Levin's definition; Leibold 1995), the breadth of resources used by each species (species niche breadth) and more particularly, the level of complementarity observed between species niches in an assembled community, is expected to have an impact on how species diversity affects ecosystem processes. Indeed, resource partitioning is considered as one of the main mechanisms describing species coexistence (resource-ratio theory, Tilman 1982) and has been regarded as a major mechanism explaining the positive effects of diversity in BEF experiments (Loreau and Hector 2001). However, the latter is based on indirect evidence supported by the additive partitioning, when the yields of communities with multiple species are on average higher than expected on the basis of the yield of their monocultures (Loreau and Hector 2001). In order to provide direct and mechanistic evidence of niche differentiation, a possible approach would be to define the niche breadths for each species present in an assemblage, and use these data to characterize the niche of that community. Whereas the concept of community niche has been used in previous ecological studies mainly focusing on trophic interactions in guilds or on factors limiting the species richness in biological communities, it has never been fully exploited in the context of the BEF within trophic levels (Leibold 1995).

Here we report the results of microcosm experiments where we used bacteria species as a model to explore whether niche differences among species provide a good estimation of community functioning. The value of bacterial model systems for testing ecological theories has been recently underlined (Jessup et al. 2004, Prosser et al. 2007), in particular for unravelling the mechanisms determining BEF relationships. Bacteria are key players in nitrogen, carbon, phosphorus and sulphur cycles; and resource-driven interactions among bacterial species are likely to have a great impact on community and ecosystem functioning (Naeem et al. 2000). In addition, for technical and biological reasons, characterizing niche breadth for a range of species can be easier for bacteria than for higher organisms.

For our experiments, we focused on bacterial species involved in denitrification, an important process in nitrogen cycling which is carried out by microorganisms that differ in their affinities for and rates of processing different substrates (Cavigelli and Robertson 2001, Philippot and Hallin 2005). We opted for the use of microcosm experiments with bacterial communities because of the simplicity of these systems which allows a full description of species performance across a range of well-defined single-resource environments and consequently the characterization of species niche breadths. These species niche breadths may allow defining the niche breadth of communities and furthermore, predicting their performance in mixed-resource environments. In this context, our objectives were to assess (1) whether niche breadths of species could be used to compute an index that represents the niche breadth of a community, and (2) whether this index would provide a better understanding of the role of species diversity on the functioning of a community. Our hypothesis was that a measure of community niche should represent the overall potential of the community to extract resources from the environment and should therefore be more directly related to ecosystem functioning than are measures of species diversity.

In order to test our hypothesis, we run two experiments. The first one consisted in determining the performance of a set of denitrifying species, growing alone on individual carbon sources. Because the bacterial species used are commonly found in terrestrial ecosystems, we selected carbon sources which also occur abundantly in soil, either due to organic matter degradation or plant exudation. The results from this experiment provided us the niche breadths for each species studied. In the second experiment we evaluated the effect of species diversity on community functioning by manipulating bacterial communities at different levels of species richness, and growing them on a mixture of the carbon sources used in the first experiment. We calculated the community niche for each bacterial assemblage by taking the sum over all carbon sources of the maximal performance observed for a species in the assemblage on each individual carbon source. Thus, consider an assemblage of two species X and Y, growing in a mixture of two carbon sources A and B. If when growing on individual substrates, species X has the highest performance on the first source, and species Y the highest on the second source, then the community niche of this assemblage (on this resource mixture) would be the sum of the performance of species X on source A and the performance of species Y in source B. Once community niche was calculated for each one of the bacterial assemblages, it was used in statistical models, together with species richness and initial species composition, to determine their importance in explaining the overall community performances observed in the second microcosm experiment.

As our results will show, species richness had a positive but small effect on community processes whereas community niche explained a much larger proportion of the variation. Thus, by aggregating resource-use characteristics of species in a community into a synthetic descriptor of community niche, we were able to better predict the observed variations in community functioning. In addition to community niche, the presence of particular bacterial species also

influenced community functioning, indicating that other effects than the capacity to use the carbon sources provided in culture medium played a role in our experiment. This study demonstrates the importance of complementarity for resource use among species in explaining the enhanced performance of diverse bacterial communities.

METHODS

Bacterial species

The 16 bacterial species used in our microcosms were chosen according to: (i) their ability to denitrify; (ii) their common occurrence in the soil or rhizosphere; and (iii) their wide distribution over different bacterial phyla. The bacterial species were identified by sequencing their respective 16S rRNA gene, which is broadly used for taxonomical purposes, and comparing it against a public database (NCBI, http://www.ncbi.nlm.nih.gov). Thus, the bacterial species belonged to the following taxa: *Achromobacter xylosoxidans* subsp. *denitrificans* D35; *Azospirillum lipoferum* A5; *Bacillus cereus* A19; *Bacillus weihenstephanensis* A20; *Burkholderia cepacia* G7; *Citrobacter braakii* A7; *Ensifer adhaerens* A1; *Klebsiela pneumoniae* A18; *Ochrobactrum* sp. A6; *Ochrobactrum* sp. A17; *Ochrobactrum* sp. A22; *Paracoccus denitrificans* G11; *Pseudomonas aeruginosa* G16; *Pseudomonas fluorescens* A14; *Pseudomonas stutzeri* A16; *and Pseudomonas stutzeri* A24. Species assigned to the same taxon were further characterized by BOX-PCR, according to the methodology described by Bathe et al. (2006) (Appendix A). The BOX is a repetitive DNA element which is present at strain-specific intergenic positions throughout the genomes and therefore can be used to generate genomic fingerprints (barcodes) that allow the classification of bacterial isolates at species, subspecies and strain level. By using this method we were able to determine that bacterial isolates assigned to the same species by sequencing the 16S rRNA gene were indeed different strains.

Microcosm experiments

The experimental microcosms consisted of 150-ml plasma flasks sealed with rubber stoppers and containing 50 ml of minimal medium M9 (Sambrook et al. 1989) supplemented with 0.2M KNO_3 and selected carbon sources (total C concentration of 1.6 mg C l^{-1}). We replaced the atmosphere of the flasks by a 90:10 mixture of He-C_2H_2 to provide anaerobic conditions for denitrification and to inhibit of N_2O-reductase activity, allowing us to quantify the amount of N_2O and CO_2 produced by the bacterial species or species assemblages. Flasks were incubated at 28°C and 160 rpm. In the microcosms used to define the species niche breadth, each bacterial species was grown on flasks containing individual carbon sources. Six sources were used, D-cellobiose, maltose, L-fucose, L-malic acid, L-glutamine and fumaric acid. They were chosen because of their occurrence in soil, where the selected bacterial were originally obtained from. The bacterial inoculum consisted of cells at the exponential growth phase, growing anaerobically on the same medium containing all six carbon sources. Before inoculation, cells were harvested by centrifugation, washed with sterile PBS buffer and let to starvation for 2 h at room temperature on PBS. The starvation step was necessary to allow cells to consume their resources completely prior to inoculation on individual sources, and avoid biases when characterizing species performances. Species were inoculated in the single C-source microcosms to obtain a final cell density of OD_{560}=0.002. This experiment was carried out twice.

The experiment used to evaluate the effect of bacterial diversity on community functioning was performed in a similar manner except for two points. The first point relates to the carbon-resource environment, which in this case corresponded of a mixture with equal C-concentration (final concentration: 1.6 mg C l^{-1}) of the six carbon sources used for the previous experiments. The second point concerns the inoculum density, which was kept at OD_{560}=0.002 for the total

community in a microcosm as previously, leading to a decrease in per-species inoculum with increasing species richness. Thus, each microcosm was inoculated with denitrifying bacterial species at a cell density of OD_{560}=0.002/*s*, where *s* is the number of species in the community inoculum. Therefore, at the beginning of the microcosm experiments, all bacterial assemblages contained approximately the same total cell density, regardless of their species richness and composition.

Measurements

The performances of individual species and bacterial communities in the respective microcosm experiments were determined by measuring both N_2O and CO_2 production (in µg-N or µg-C ml^{-1} h^{-1}), which will be referred to throughout the text as denitrification and (anaerobic) CO_2-production, respectively. Both measurements were performed, after collecting gas samples on regular basis up to 8 days after inoculation, using a gas chromatograph (Agilent P200, USA). In addition, at each sampling point, we collected 1 ml of culture, which we used to determine the total number of bacterial cells with a flow cytometer (BD FACSCalibur, USA), providing us with information about possible effects of species diversity or composition on the cell density in each microcosm.

Experimental design

We assembled bacterial communities following a "broken stick" design, which allows separation of the effects of bacterial richness and composition (Bell et al. 2005), because each species combination is nested in the combinations of higher species richness. This design consists in defining a list or "stick" where all 16 species are put in random order without replication. The

complete stick corresponds to the assemblage with all species. We then divided this stick in the middle, generating two sticks of 8 species that corresponded to two assemblages, one containing eight species present in one half of the stick and the other assemblage containing the remaining eight species. Each 8-species stick was further divided in the middle; creating four 4-species sticks and so on, until the 16 monocultures were reached. In order to distinguish the effects of species richness from composition, it is essential that for a given level of species richness, assemblages with different bacterial composition are created. We therefore made three 16-species sticks, each one containing the same species but in different order. These sticks were "broken", providing 6 assemblages with 8 species, 12 with 4 species, 24 with 2 species and 16 monocultures. In order to increase the number of communities with 8 species, we added 3 extra assemblages, each consisting of the first four and the last four species of each one of the 16-species sticks. The 16 monocultures were replicated twice and the single 16-species assemblage three times, giving a total of 80 microcosms.

Community niche

The niche of a given community (community niche, CN) was calculated based on the performance of each species on each one of the carbon sources, according to the formula:

$$CN = \sum_{i=1}^{6} \max_{j=1}^{n} \left(P_{ij} \right)$$

where P_{ij} is the performance (i.e. denitrification or anaerobic CO_2-production activity, in mgN-N_2O or mgC-CO_2 ml^{-1} h^{-1}) of species *j* on carbon source *i*, and *n* is the number of species in the considered community. In other words, considering a set of species present in a community and their variety of individual performances on a range of sources, the niche from that community will correspond to sum of the best performances on each source present on the environment

where that community is functioning. We used the maximum or best performances because this is the activity level that could be achieved if the corresponding species would become dominant in the community (referred to as selection or dominance effect in biodiversity studies, see Loreau and Hector 2001, or Fox 2005, respectively). Community niche was used in the statistical analyses, together with species richness and composition to test for their effect on community functioning (see below). The performances of species on individual carbon sources used to calculate community niche are listed in Appendix B.

Data analysis

General linear models (GLMs) were used to evaluate the effects of community niche, bacterial richness (log scale), contrasts for the presence of particular bacterial taxa, composition (different combinations of bacterial taxa) and cell density on bacterial community functions at 8 days after starting the microcosms. This duration corresponds to about 13 bacterial generations (see second paragraph of Results section). Alternative models in which the richness term or the contrasts for particular bacterial taxa were fitted before community niche were also analyzed (data not shown). Selection of particular species contrasts was done by forward inclusion and backward elimination using the significance level $P = 0.05$. Results were summarized in analysis of variance (ANOVA) tables (Table 1). In these, contrasts for the presence/absence of particular species were ordered in the sequence in which they were entered into the model during the forward selection process. Path analyses were used to test causal relationships between community niche, species richness and community processes (denitrification and CO_2 production). The path analyses were calculated based on correlation matrices between explanatory terms and the dependent variables.

RESULTS

Characterization of individual bacterial species

The measurement of denitrification and anaerobic CO_2 production for each bacterial species on each of the six carbon sources individually revealed that species differed in their performances, indicating that the bacterial species were functionally diverse under the conditions studied (Fig. 1). Additional results based on BOX-PCR and 16S rRNA sequencing confirmed that these species were also genetically distinct (data not shown).

Species richness and community functioning

Eight days after starting the microcosms, both community denitrification and CO_2 production increased linearly with the logarithm of species richness of the inoculum (Fig. 2). Fitted as first explanatory term in a GLM similar to the one presented in Table 1, the log-linear effect of species richness explained 14 % ($P = 0.0014$) and 18 % ($P = 0.0004$) of the variance for community denitrification and anaerobic CO_2 production, respectively. The average generation times of bacterial species in the monocultures from the assemblage experiment was 20.3 ± 2.9 h (mean ± s.e.m), indicating that the bacterial communities on average had turned over 13.2 ± 2.2 times during this time period. Thus, the positive relationship observed between initial richness and community processes reflected a long-term outcome after multi-generation interactions between and within species. As a consequence, community composition might have changed during the experiment and thus realized species richness at the end of the experiment might have differed from initial species richness. Although realized richness could not be assessed in this study, we did measure total bacterial abundance at the end of the experiment and found that this covariate did not influence the measured community processes, denitrification and CO_2 production (Table 1).

Effects of community niche versus other components of biodiversity on functioning

The performances of species on individual carbon sources were used to calculate community niche for denitrification (CN_{N2O}) and for anaerobic CO_2 production (CN_{CO2}), for each bacterial assemblage used as inoculum in the experiment. A positive linear relationship was observed between community niche and the two community processes (Fig 3), indicating the importance of the observed complementarity in resource use capacity among species. Fitting community niche before species richness in the GLM demonstrated that CN_{N2O} and CN_{CO2} fully accounted for the positive effects of species richness on community denitrification and CO_2 production, respectively (Table 1). Moreover, the explanatory power of the community niche terms was much larger than that of richness if these terms were each fitted first in separate GLMs: 46 % of the variance for denitrification and 32 % of the variance for CO_2 production was explained by CN_{N2O} and CN_{CO2}, respectively (Table 1). It is interesting to notice that there was only a weak overlap among the functionally important species identified during the GLM analyses for denitrification versus CO_2 production (Table 1).

Graphical examples of interactions between species are provided in Figure 4. Interactions between species were most often found to be positive and explained by the pattern of resource utilization (Fig. 4c) whereas negative interactions were also observed (Fig. 4d) although less frequently. It should be noted, however, that these results are based on single observations, as assemblages with the same composition were not replicated. Our results thus provide indication on the role of species interactions, but further experiments are needed to quantify this role.

Community niche and species contrasts together explained 68 % and 69 % of the variation in denitrification and CO_2 production, respectively. When community niche was fitted after species richness, community niche still explained 30 % and 13 % of the variation in community

denitrification and CO_2 production, respectively. When CN_{N2O} was used as an explanatory term in the GLM for community CO_2 production, or CN_{CO2} as an explanatory term in the GLM for community denitrification, their explanatory power was more than halved, demonstrating that the best predictor for a particular process is indeed the measure of community niche directly associated with that process.

The relationship among the different components of biodiversity and the measured community processes was evaluated by path analysis (Fig. 5). For both processes, the effect of species richness operated mainly through community niche. When entered after community niche into the statistical model, richness had a weak negative and positive effect on denitrification and CO_2 production, respectively.

DISCUSSION

In this study we took advantage of the tractability of the bacterial model systems to evaluate the role of niche differentiation among species for biodiversity–ecosystem functioning relationships (Leibold and McPeek 2006). We hypothesized that by defining an index for the breadth of the niche occupied by a community, here called community niche, we should be able to predict ecosystem processes which are related to resource extraction by that community in a given multiple-resource environment.

The question of whether bacterial diversity matters to ecosystem processes has been previously addressed (Wohl et al. 2004, Bell et al. 2005, Wertz et al. 2006, Jiang 2007, Wertz et al. 2007), and the results range from negative or neutral to positive relationships. Jiang et al. (2008) have recently argued that a positive relationship between biodiversity and ecosystem functioning may not be a general trend. They suggested that negative selection (where competitively dominant species do not contribute significantly to the function of interest) is more

likely to operate for non-biomass ecosystem processes, leading thus to neutral or even negative biodiversity–ecosystem functioning relationships (Jiang 2007, Jiang et al. 2008). Our findings do not support this hypothesis, as species richness did have a positive effect on denitrification and CO_2 production, two ecosystem processes not entirely dependent on biomass production. Moreover, denitrifying bacterial species differ greatly with respect to their specific activities (i.e. activity per cell), and an indication of these differences can be seen in Fig. 2, where large variations in denitrification (and CO_2 production) are observed for the monocultures. Although these values do not represent the specific activities of the cultures, they give an indication of it, because cell density did not significantly vary among cultures (GLM analysis). A similar reasoning can be applied for CO_2 production, where species might have different catabolic abilities. Given our findings, we suggest that additional bacterial biodiversity–ecosystem functioning experiments, targeting a range of functions, are necessary to determine whether and which generalizations can be made.

Despite its significance, species richness had a relatively low explanatory power in our study, which is typical for a large number of biodiversity experiments (Balvanera et al. 2006, Weigelt et al. 2008) and indicates that further components of biodiversity affect the variance in bacterial community processes. This was confirmed by including community niche in the analysis, which greatly expanded the explained variance. An additional part of the variation in community processes could be explained by the presence or absence of particular species, indicating that effects other than the capacity to use the carbon sources also played a role in our experiment, as exemplified in Fig. 4d. However, identifying the mechanisms generating the effects of particular bacterial species (production of antibiotics, quorum sensing signals, etc.) was beyond the scope of this study. Furthermore, it is conceivable that species behaved differently in mixtures than in monoculture and this might be an additional reason for unexplained variance in

our experiment. Finally, as time went by in this multi-generation experiment, other resources may have become available through the release of bacterial metabolites or due to cell death. Nevertheless, path analysis demonstrated that community niche was the main driver for the corresponding community process and provided evidence that species richness influenced denitrification and CO_2 production only indirectly via its influence on community niche.

The positive linear relationships between community niche and functioning for both processes studied indicated that the larger the community niche of a given community, the more efficient it can exploit the available resources and the better it performs in the corresponding process. This finding led us to the conclusion that the pattern of resource utilization by individual species and among species was the major effect explaining enhanced collective performance of diverse communities. Attempts to provide a mechanistic interpretation of bacterial diversity-ecosystem functioning experiments suggest that complementarity (through resource partitioning and/or facilitation) plays a more important role than selection effect, when functions related to biomass production were evaluated (Wohl et al. 2004, Bell et al. 2005, Jiang 2007). An open question is whether it is relevant to disentangle the role of selection and complementarity in multi-generation biodiversity experiments. We argue that for microbial communities, widely-used mechanistic interpretation of biodiversity–ecosystem functioning such as those based on additive partitioning methods (Loreau and Hector 2001, Fox 2005) seem less appropriate since population dynamics leads to potentially strong shifts in individual species abundances along a time scale. Moreover, in these experiments, niche partitioning leads to transient dynamics which reflects evolutionary responses resulting from both selection and complementarity effects. We propose that in the case of dynamic communities, indices based on the effects of species on ecosystem processes are more appropriate as they go beyond these interpretations, and yet provide a mechanistic explanation. This is the case for community niche, but also indexes based

on the functional dissimilarity between species (Heemsbergen et al. 2004). All these indices could be used, due to their deductive reasoning, to predict how communities influence ecosystem processes prior to or without experimentation.

Our conclusions cannot be directly generalized to natural environments often characterized by very large bacterial diversity that can lead to high functional redundancy (Wertz et al. 2006, 2007). Rather, our study provides a proof of concept how measures of community resource use may be used to predict ecosystem functioning under ideal conditions. It remains to be tested how well such predictions will match with the more complex situation in the field. Although we used bacteria to test our hypothesis, our approach could be extended to other organisms such as plants, for which the best choice of functional diversity measures remains a challenge (Lavorel and Garnier 2002, Naeem and Wright 2003, Petchey and Gaston 2006). For instance, the characterization of the uptake rates of different nitrogen compounds in soil such as nitrate, ammonium and small organic nitrogen compounds by a range of plant species could be used to determine a community niche for different plant communities and predict plant diversity effects on ecosystem processes such as overall N uptake from soil or primary production. Furthermore, for experiments reflecting short-term dynamics, the already high explanatory power of this index might be further enhanced if the performance of each species could be weighed by its actual abundance in each community.

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Table 1. Results of GLM analyses presenting the effects of community niche, species richness, contrasts for the presence of particular species, community composition (different combinations of species) and cell density on bacterial community functions 8 days after inoculating the microcosms. Significant terms are in bold.

Denitrification	d.f.	*F*	*P*	% s.s.	CO_2 production	d.f.	*F*	*P*	% s.s.
Community niche	**1**	**99.72**	**0.0000**	**46.22**	**Community niche**	**1**	**66.47**	**0.0000**	**31.90**
Species richness	1	0.44	ns	0.20	Species richness	1	1.44	ns	0.69
***Citrobacter braakii* A7**	**1**	**14.58**	**0.0004**	**6.76**	***Pseudomonas aeruginosa* G16**	**1**	**10.47**	**0.0021**	**5.03**
***Ochrobactrum* sp. A17**	**1**	**9.28**	**0.0036**	**4.30**	***Ochrobactrum* sp. A6**	**1**	**16.61**	**0.0002**	**7.97**
***Pseudomonas stutzeri* A24**	**1**	**9.09**	**0.0040**	**4.21**	***Ensifer adhaerens* A1**	**1**	**13.27**	**0.0006**	**6.37**
***Ochrobactrum* sp. A22**	**1**	**6.03**	**0.017**	**2.80**	***Ochrobactrum* sp. A22**	**1**	**16.17**	**0.0002**	**7.76**
Pseudomonas aeruginosa G16	1	3.06	0.0847	1.43	***Bacillus weihenstephanensis* A20**	**1**	**4.25**	**0.044**	**2.04**
***Klebsiela pneumoniae* A18**	**1**	**4.49**	**0.039**	**2.08**	***Pseudomonas stutzeri* A24**	**1**	**8.12**	**0.0063**	**3.90**
					***Azospirillum lipoferum* A5**	**1**	**8.27**	**0.0059**	**3.97**
Total species	**6**	**7.76**	**0.0000**	**21.58**	**Total species**	**7**	**11.02**	**0.0000**	**37.02**
Composition	52	1.10	ns	24.10	Composition	51	1.63	ns	24.48

Cell density	1	1.81	ns	0.76	Cell density	1	3.21	ns	0.95

Note that species contrasts cannot be ordered in sequence of decreasing explanatory power because some species explain more variation if they are fitted after than before other species. Therefore, we used the sequence in which species were entered into the model during the forward selection process. d.f. = degrees of freedom. % s.s. = percentage of the total sum of squares explained by each variable. ns = not significant ($P > 0.05$).

FIGURE LEGENDS

Figure 1: PCA analysis based on the relative performance (N_2O production) of each species on each carbon source. Diamonds represent the bacterial species; arrows are projected vectors corresponding to carbon sources: C1, D-cellobiose; C2, maltose; C3, L-fucose; C4, L-malic acid; C5, L-glutamine and C6, fumaric acid. The first and the second axes accounted for 52.4 and 17.8% of total variance, respectively.

Figure 2: Relationship between the functioning and species richness of bacterial communities. Two processes were studied: (a) denitrification and (b) CO_2 production under anaerobiosis. Each diamond represents a single community, and asterisks represent mean values at each richness level. Regression lines are linear fits with log (species richness) as independent variable, excluding the highest richness level (16 species of the initial pool). Note that two points are confounded for the 16 species level.

Figure 3: Relationship between community functioning and community niche. Community niche was rescaled by dividing by the maximum value observed for all communities: (a) denitrification as a function of community niche defined according to monoculture ability to perform denitrification on each individual carbon source; (b) anaerobic CO_2 production as a function of community niche defined according to monoculture ability to produce CO_2 on each individual carbon source. Different colors correspond to different levels of species richness. Each symbol corresponds to a single community, except for the monocultures, which are represented by their average performances. The graph shows that communities with low richness but large community niche perform better than communities with high richness but small community niche.

Figure 4: Example of complementarity and of negative interaction between species. Complementarity was observed between two species (G11: *Paracoccus denitrificans* and G16: *Pseudomonas aeruginosa*) that differ in their ability to produce N_2O on different carbon sources (a); in that case, the performance of the mixture of the 2 species (grey diamond) is higher than the performance of each monoculture (black diamonds) which is adequately predicted by the change in community niche (c). Negative interaction was observed between two other species (A20: *Bacillus weihenstephanensis* and A24: *Pseudomonas stutzeri*). In that case, independently of the differences in their ability to produce N_2O on different carbon sources (b), the performance of the mixture of the 2 species is lower than the performance of each monoculture (d) which indicates negative interactions such as antibiotics or inhibitory compounds production.

Figure 5: Relative effect of bacterial richness and community niche on community functioning. Path analyses were used to evaluate the roles of these different aspects of biodiversity for (a) denitrification and (b) anaerobic CO_2 production. The values not linked to any variable indicate that amount of variance that remains unexplained.

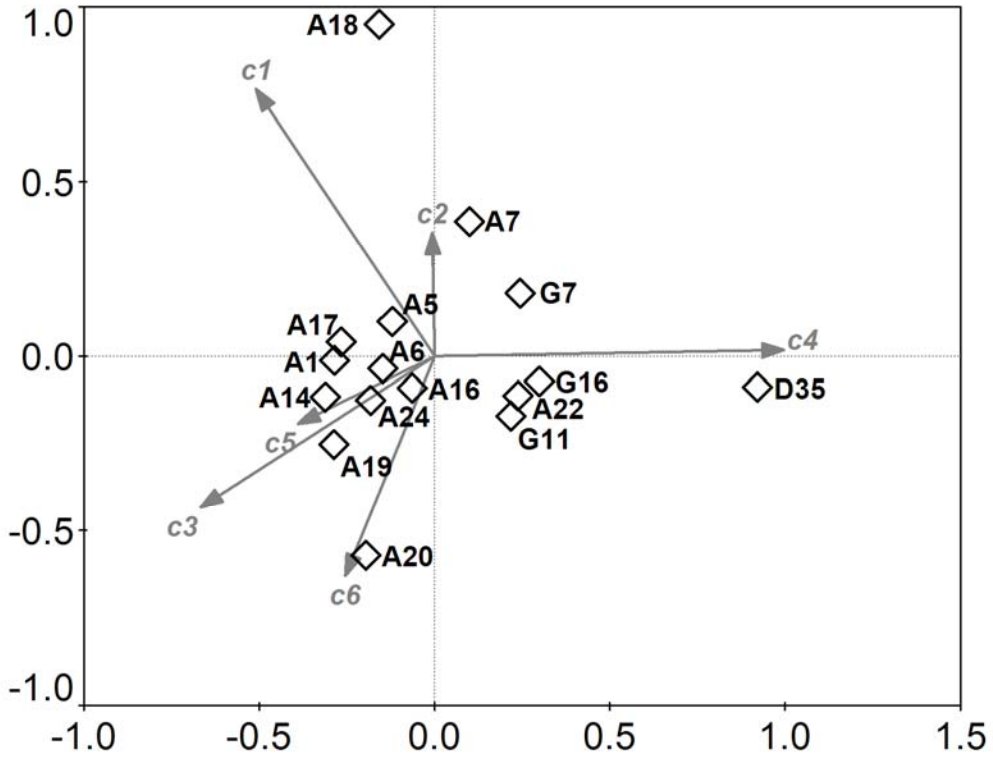
1.0
0.5
0.0
-0.5
-1.0
-1.0
-0.5
0.0
0.5
1.0
1.5
A18
c1
c2
A7
G7
A5
A17
A1
A6
c4
A14
A16
G16
D35
A24
A22
G11
c5
A19
c3
A20
c6

Figure 1

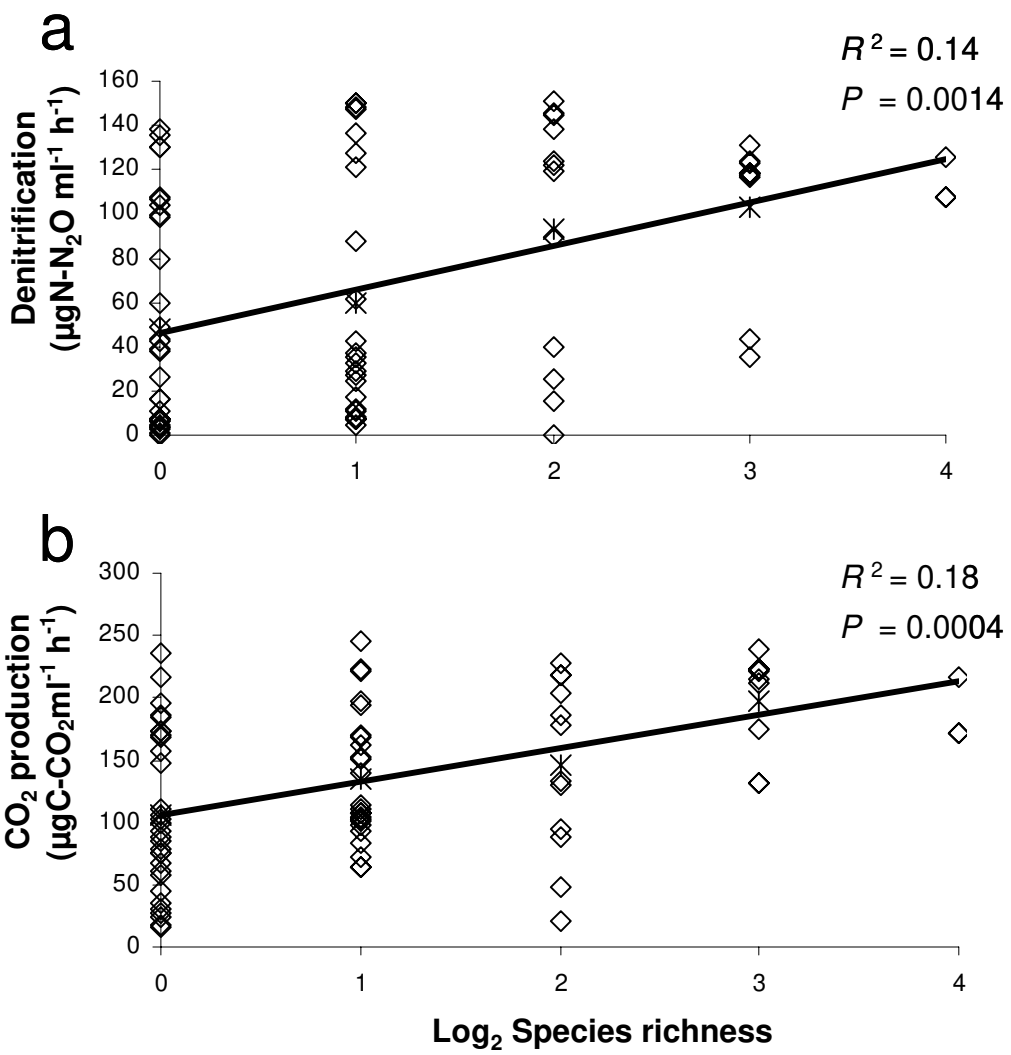
a
R^2 = 0.14
P = 0.0014
Denitrification (µgN-N_2O ml^{-1} h^{-1})
160
140
120
100
80
60
40
20
0
0
1
2
3
4
b
R^2 = 0.18
P = 0.0004
CO_2 production (µgC-$CO_2$$ml^{-1}$ h^{-1})
300
250
200
150
100
50
0
0
1
2
3
4
Log$_2$ Species richness

Figure 2

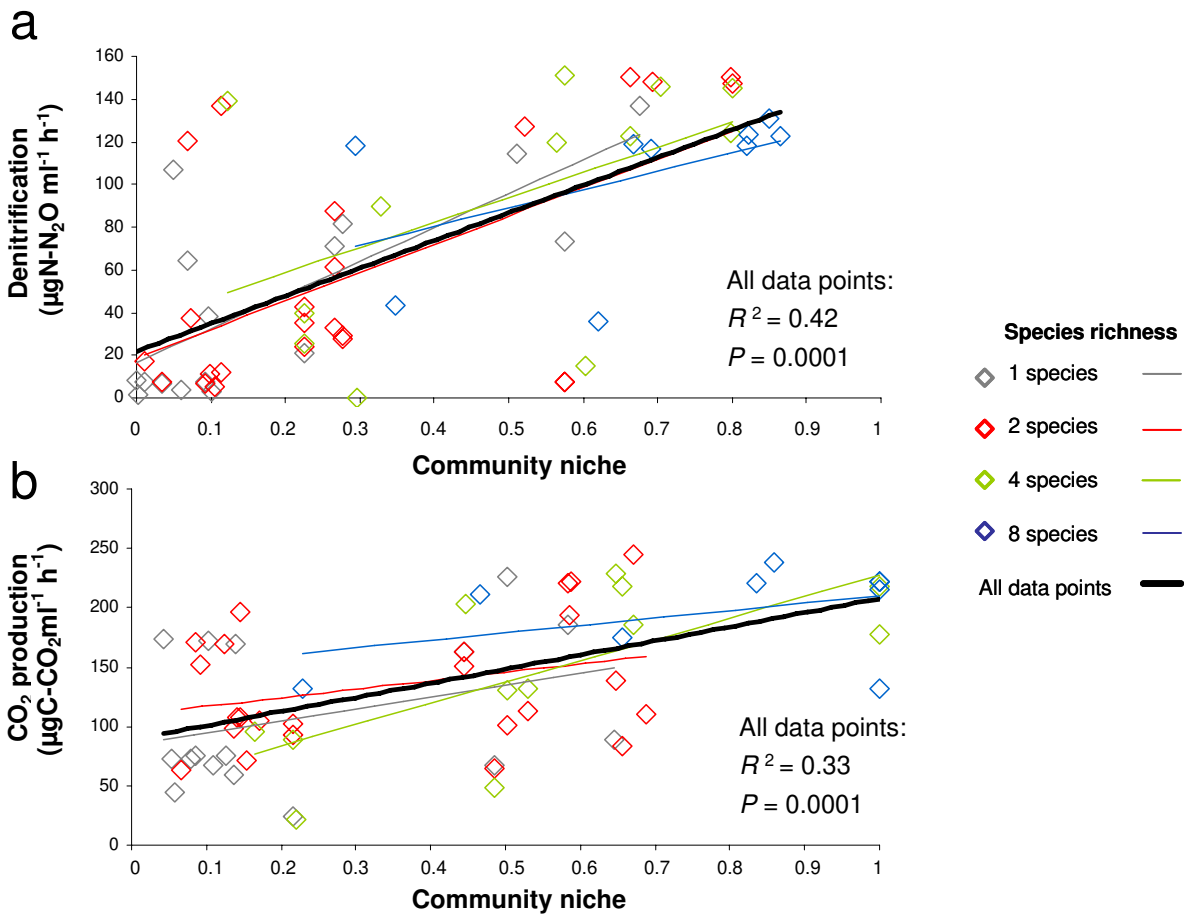
a
Denitrification (µgN-N2O ml-1 h-1)
160
140
120
100
80
60
40
20
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Community niche
All data points:
R 2 = 0.42
P = 0.0001
b
CO2 production (µgC-CO2ml-1 h-1)
300
250
200
150
100
50
0
Community niche
All data points:
R 2 = 0.33
P = 0.0001
Species richness
1 species
2 species
4 species
8 species
All data points

Figure 3

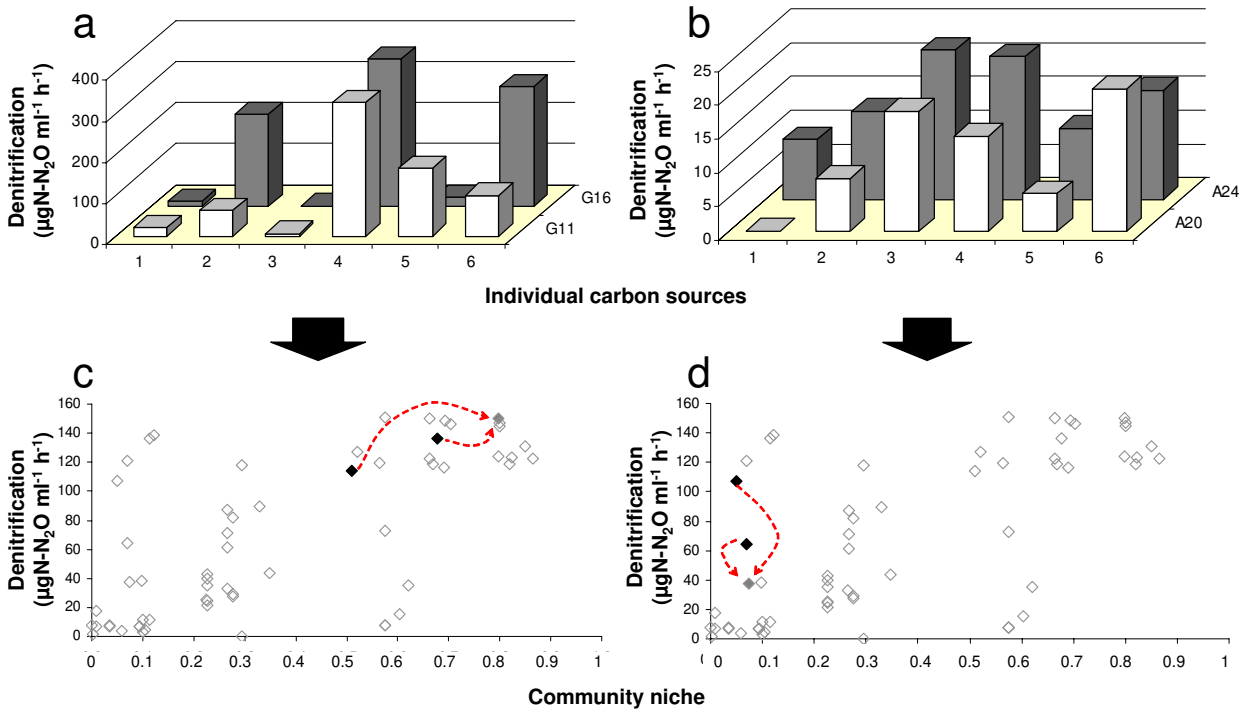
a
b
c
d
Denitrification (µgN-N2O ml-1 h-1)
G16
G11
A24
A20
Individual carbon sources
Community niche

Figure 4

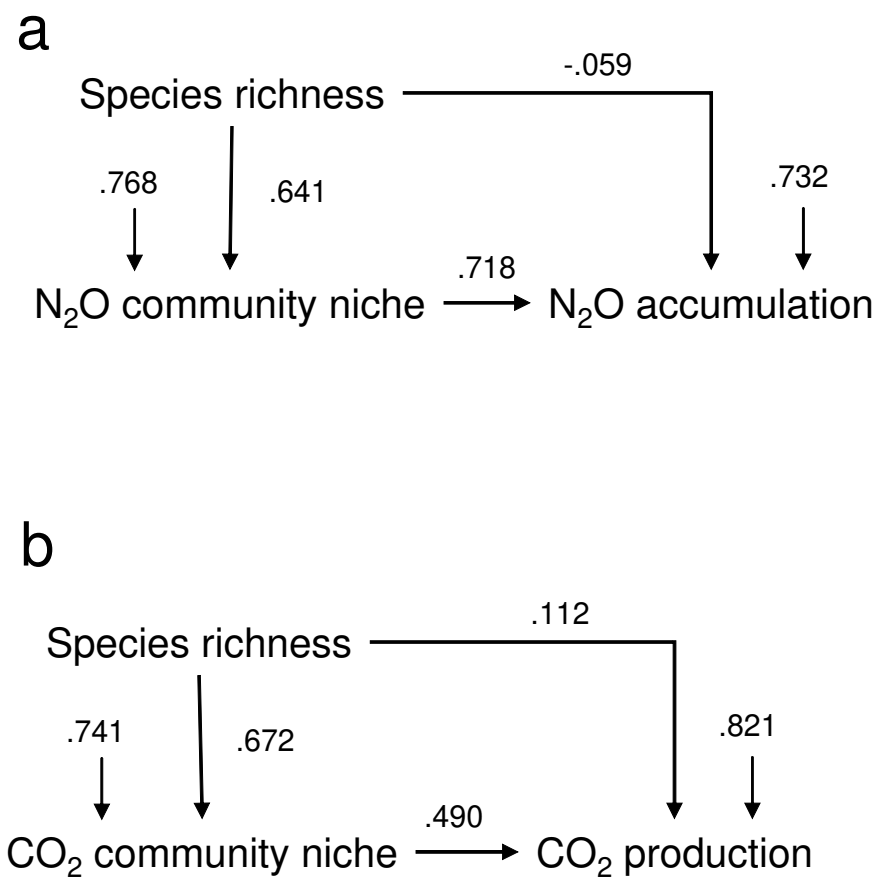
a
Species richness
-.059
.768
.641
.732
.718
N_2O community niche
N_2O accumulation
b
Species richness
.112
.741
.672
.821
.490
CO_2 community niche
CO_2 production

Figure 5